MOLECULAR PHYLOGENETICS AND MORPHOLOGICAL EVOLUTION IN CUNONIEAE (CUNONIACEAE)[1]

Jason C. Bradford[2]

Abstract

The Cunonieae are the largest tribe in the flowering plant family Cunoniaceae and include the widespread genus *Weinmannia*. This study aims to understand phylogenetic relationships within Cunonieae by using DNA sequences in a parsimony-cladistic analysis. Sequenced loci included the internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA, and the *trn*L intron and *trn*L-F spacer of chloroplast DNA. Primer and taxon-specific amplification of non-orthologous ITS-2 copies made it necessary to exclude the ITS-2 data, but otherwise the nuclear and chloroplast data sets were congruent. The results place *Vesselowskya* as the sister genus to other Cunonieae and support the monophyly of *Pancheria*, *Cunonia*, and all five sections of *Weinmannia*, but do not indicate how these groups are related. The monophyly of *Weinmannia* sect. *Weinmannia* is upheld, with *W. trichosperma* from temperate forests of South America and *W. tinctoria* from the Mascarene Islands placed basal to a large clade of tropical American species. Although morphological data maintain the monophyly of *Weinmannia*, this is neither verified nor statistically refuted by the molecular data. Likewise, *Cunonia*, with one isolated South African species, has only weak molecular support but clear morphological synapomorphies. Lack of support for relationships among major clades within Cunonieae makes it difficult to suggest patterns of morphological evolution. However, a well-supported phylogenetic hypothesis within *Weinmannia* sect. *Leiospermum* is used to discuss heterotopy in inflorescence architecture. Uniquely derived features of the inflorescence are found in the New Caledonian species *Weinmannia dichotoma* and in the New Zealand species *W. silvicola* and *W. racemosa*. These heterotopic changes involve alternate patterns in the fate of terminal meristems and the arrangement of metamers bearing racemes. In an appendix the correct orthographies and original publications of all five sections of *Weinmannia* are provided; types are also designated for *Weinmannia* sections *Inspersae* and *Spicatae* in order to validate them.

Key words: cladistics, *Cunonia*, Cunoniaceae, Cunonieae, evolution, *Fasciculatae*, heterotopy, inflorescence architecture, *Inspersae*, ITS, *Leiospermum*, molecular systematics, *Pancheria*, paralogous loci, *Spicatae*, *trn*F, *trn*L, *Vesselowskya*, *Weinmannia*.

The flowering plant family Cunoniaceae R. Br. (Oxalidales) (Angiosperm Phylogeny Group, 1998) comprises about 300 species in 26 genera. Plants of the family are trees and shrubs in wet tropical and cool temperate forests, with most genera occurring in eastern Australia, Melanesia, and New Guinea. About 210 Cunoniaceae species are in a monophyletic group of four genera called the tribe Cunonieae (Bradford & Barnes, 2001). *Weinmannia* is by far the largest and most widely distributed genus, with over 150 species found in the Americas, islands of the eastern Indian Ocean, Malesia, and the South Pacific (Bradford, 1998; Hopkins, 1998a). *Weinmannia* is divided into five sections, with each one largely restricted to a particular geographic region. The Cunonieae are also composed of the two other largest genera in Cunoniaceae, *Pancheria*, with about 30 species endemic to New Caledonia (Guillaumin, 1940; Morat, 1993), and *Cunonia*, with about 25 species in New Caledonia and 1 species in the South African Cape region (Hoogland et al., 1997). *Vesselowskya*, the remaining genus in the tribe, has only two species endemic to eastern Australia (Rozefelds et al., 2001).

A few recent publications have provided new insights on the taxonomy and phylogeny of Cunon-

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ieae. In a study of the relationship between *Weinmannia* and its putative close relative, *Cunonia*, using a cladistic analysis of morphological features (Bradford, 1998) there was weak support for the monophyly of *Weinmannia*, four of its sections, and of *Cunonia*. A family-level analysis by Bradford and Barnes (2001) based on morphology and chloroplast DNA sequences (*rbc*L and *trn*L-*trn*F) established a new tribal classification, and clarified generic circumscriptions by proposing apomorphic morphological characters for each genus.

Several recent generic revisions have provided valuable details on taxonomic distribution and morphology for many Cunonieae species. Rozefelds et al. (2001) gave a table summarizing similarities and differences among genera of Cunonieae and described a new species of *Vesselowskya*. New species descriptions and keys have also been produced for *Cunonia* (Hoogland et al., 1997) and Malagasy *Weinmannia* (Bradford, 2001; Bradford & Miller, 2001). Revisions have been completed for *Weinmannia* of Malesia and the South Pacific (Hopkins, 1998a, b, c; Hopkins & Florence, 1998). *Weinmannia* of the Americas are poorly studied in their entirety, although some national and regional treatments have been done (Harling, 1999; Bradford & Berry, 1998).

Despite these efforts, relationships among Cunonieae genera are unclear, the monophyly of *Weinmannia* is in doubt, and the monophyly of some sections within *Weinmannia* is poorly established. In this study, I use DNA sequences from chloroplast and nuclear loci to clarify phylogenetic relationships within Cunonieae. In addition, I show that the phylogenetic hypotheses generated by this molecular data can help reevaluate character evolution within the tribe, especially with respect to inflorescence architecture.

Methods

Based on the family-level analysis of Bradford and Barnes (2001), Cunonieae are clearly monophyletic, and taxon sampling was designed to maximize the geographic, phylogenetic, and morphological diversity within this clade that has been elucidated by previous studies (Bradford, 1998). One distinctive species endemic to Sulawesi, *Weinmannia descombesiana*, is missing; otherwise, sampling is broad, including exemplars from 45 species of Cunonieae (Table 1). Codieae may be the most closely related tribe to Cunonieae, but I was unable to obtain internal transcribed spacer (ITS) nrDNA sequences of Codieae to use as outgroups. Instead, ITS trees were rooted using two species from another closely related tribe, Caldcluvieae (Bradford & Barnes, 2001). The *trn*L-*trn*F cpDNA (Taberlet et al., 1991) data set uses several outgroup taxa from closely related tribes.

Collections were made from native populations and botanical gardens between 1994 and 1998. Fresh leaves were dried in silica gel for DNA preservation. All DNA samples are vouchered with herbarium specimens and were deposited at MO and in the country of origin. Table 1 lists source and voucher information of each herbarium specimen and GenBank accession numbers for all DNA sequences. Detailed information is available for Bradford collections on the TROPICOS database (⟨http://www.mobot.org⟩).

I sequenced both the nuclear ITS region (Baldwin, 1992) and two adjacent chloroplast loci, the *trn*L intron and the intergenic spacer between the *trn*L 3′ exon and *trn*F (Taberlet et al., 1991). The ITS region was sequenced first, which helped establish likely monophyletic groups. A smaller set of *trn*L-*trn*F sequences was obtained from a subsample of each distinct lineage that was discerned from ITS data. Standard methods were used to extract, amplify, and sequence DNA loci, and these are described in Bradford and Barnes (2001). BLAST (⟨http://www.ncbi.nlm.nih.gov/BLAST/⟩) comparisons were done to confirm that sequences were of angiosperm origin and not from possible fungal or other contaminants, and indeed similar nucleotide sequences were of appropriate loci and within the eudicot clade.

Standard ITS primers (Baldwin, 1992) did not strongly amplify or produce clear sequences of ITS-2 for many species in *Weinmannia* sections *Leiospermum* and *Inspersae*, or in *Pancheria*, *Geissois*, and *Caldcluvia*. Hypothesizing that high G-C content was interfering with PCR amplification, I designed alternative primers with higher annealing temperatures based on published sequences of 26S rDNA (Kuzoff et al., 1998) and 5.8 rDNA sequences from my own work. The new primers did yield clear sequences as hoped, but preliminary cladistic analysis using ITS-2 data alone resolved clades with a mixture of ingroups and outgroups, a result incongruent with ITS-1, chloroplast, and morphological data (see Bradford, 2000, chapter 1, for a figure of these results). This suggested that the ITS-2 region amplified using the new primers was not orthologous to ITS-1 sequences obtained using the standard (e.g., ITS4) primers. To test this, a Partition Homogeneity Test (PAUP*4.0b6a; Swofford, 2001) was used to compare the ITS-1 and ITS-2 data sets, and they were found to be significantly incongruent (500 replicates, $P = 0.002$). Because

ITS-1 and ITS-2 are adjacent loci they should have the same evolutionary history; significant incongruence between them strongly suggests that non-orthologous loci were amplified. The ITS region is known to contain multiple copies of the ribosomal genes, as well as pseudogenes, and different PCR conditions can preferentially amplify different paralogous loci (Buckler et al., 1997). Until the issue of paralogy can be resolved, the decision was made to exclude the ITS-2 data from analyses of organismal phylogeny; these sequences, however, are available in GenBank (phylogenetic data set with the range AF521255–AF521298).

Sequences were aligned by eye in Se-Al (Rambaut, 1995) and exported in a NEXUS format. Insertions and deletions were scored as binary characters (e.g., present or absent). Any regions with ambiguous sequence or uncertain alignment were ignored during analysis. Parsimony cladistic analyses were implemented in PAUP*4.0b6a (Swofford, 2001). For all parsimony analyses, the following options were used: characters unweighted and unordered, searches heuristic, starting trees obtained via random stepwise addition, TBR branch swapping, COLLAPSE option on, STEEPEST DESCENT option off, MULTREES on. Support for clades was estimated with bootstrap values (using the "Fast" bootstrap option with 10,000 replicates in PAUP) and decay values (using Autodecay; Eriksson, 1999).

The nuclear and chloroplast data sets were combined after checking for compatibility using the Partition Homogeneity Test in PAUP. This test could not reject the null hypothesis that the data sets represent the same evolutionary history (500 replicates, $P = 0.06$).

Results

ITS-1 analysis

The final ITS-1 data set included 48 sequences from 47 species (45 in Cunonieae) and a matrix of 260 characters. The number of equally parsimonious trees found during heuristic searches made it impossible to complete branch swapping. Several searches were done using random taxon addition, and each analysis found the same large tree island with 268 steps (CI = 0.70, RI = 0.86). The strict consensus of this tree island shown in Figure 1 is based on over 30,000 equally parsimonious trees.

The ITS-1 data strongly support the sister-group relationship between *Vesselowskya* and the rest of Cunonieae (Fig. 1). *Pancheria* is monophyletic and weakly placed as the sister to clades of *Weinmannia* and *Cunonia*. Five traditionally recognized groups are resolved as clades, including *Cunonia*, and four sections of *Weinmannia*: sects. *Weinmannia*, *Inspersae*, *Spicatae*, and *Fasciculatae*. Species from *Weinmannia* sect. *Leiospermum* form part of a large polytomy. The two sections from Madagascar, section *Inspersae* and section *Spicatae*, are placed as sister taxa to each other. Not all of these clades have high "Fast" bootstrap values, however, and the data give no support for relationships between these larger clades.

*trn*L-F analysis

The *trn*L-F data set included 996 characters from sequences representing 38 species (29 in Cunonieae). Ingroup sampling was less intensive than with the ITS data set, but included sufficient sampling from all major ITS clades. The parsimony analysis found 420 trees of 244 steps (CI = 0.73, RI = 0.82) (Fig. 2).

On the strict consensus tree, Cunonieae are monophyletic, and *Vesselowskya* is placed as the sister group to other Cunonieae. In contrast to ITS results, *Weinmannia* sect. *Weinmannia* is the sister taxon to a large clade containing *Cunonia*, *Pancheria*, and all other species of *Weinmannia*. *Cunonia capensis*, from South Africa, groups with *Pancheria* while other *Cunonia* are monophyletic and sister to the remaining *Weinmannia*. Malagasy *Weinmannia* (sects. *Inspersae* and *Spicatae)* form a clade, as do sections *Fasciculatae* and *Leiospermum*. The internal topology of the cladogram has low bootstrap values.

Combined analysis

For the combined analysis, 29 taxa, including 27 from the Cunonieae, were sequenced for both ITS and *trn*L-F. The final data set included 1254 characters. The parsimony analysis found a single most parsimonious tree of 388 steps (CI = 0.74, RI = 0.81) (Fig. 3).

The base of the tree is structured similar to the ITS trees: *Vesselowskya* is basal to all Cunoniae, and *Pancheria* is a sister taxon to the clade containing *Weinmannia* and *Cunonia*. Although there is strong support for the position of *Vesselowskya*, no other internal branches have high "Fast" bootstrap or decay values. High "Fast" bootstrap and decay values do support most of the commonly recognized taxa, including *Pancheria* and all five *Weinmannia* sections. By contrast, *Cunonia* does not form a clade in the "Fast" bootstrap consensus tree and has a decay value of one.

Table 1. Vouchers for exemplars used in DNA sequencing and the GenBank number of each sequence are given. Exemplars are organized by outgroups, Cunonieae, genera, and sections of *Weinmannia*. Based on recent revisions and work in progress, the best current estimates of the number of species in Cunonieae and its subtaxa are given.

Genus	Species (number of species in taxon)	Collection	Native origin (cultivated)	*trn*L c–d	*trn*L e–F	ITS1
OUTGROUPS						
Ackama	*paniculosa* (F. Muell.) Beuzev. & C. T. White	*Bradford 843* (MO, BRI)	Australia	AF299161	AF299214	
Ackama	*rosifolia* A. Cunn.	*Bradford 909* (MO, BRI)	New Zealand	AF299162	AF299215	
Caldcluvia	*paniculata* D. Don	*Barnes s.n.* (HO 516497)	Chile (Australia)	AF299163	AF299216	AF485640
Callicoma	*serratifolia* Andrews	*Bradford 857* (MO, NSW)	Australia	AF299170	AF299223	
Codia	*discolor* Guillaumin	*Bradford 600* (MO, NOU)	New Caledonia	AF299171	AF299224	
Geissois	*benthamiana* F. Muell.	*Bradford 859* (MO, BRI)	Australia	AF299165	AF299218	
Geissois	*superba* Gillespie	*Hopkins 5019* (MO, SUVA)	Fiji			
Pullea	cf. *glabra* Schltr.	*Bradford 585* (MO, SUVA)	Fiji	AF299172	AF299225	
Spiraeopsis	*celebica* Blume	*Bradford 840* (MO, BSIP)	Solomon Islands	AF299164	AF299217	AF485641
CUNONIEAE R. Br. (210)						
Cunonia L. (26)						
Cunonia	*atrorubens* Schltr.	*Bradford 614* (MO, NOU)	New Caledonia	AF299154	AF299207	AF485628
Cunonia	*balansae* Brongn. & Gris.	*Bradford 617* (MO, NOU)	New Caledonia	AF299155	AF299208	AF485629
Cunonia	*capensis* L.	*Bradford 735* (MO)	South Africa (U.S.)	AF299156	AF299209	AF485630
Cunonia	*macrophylla* Brongn. & Gris	*Bradford 607* (MO, NOU)	New Caledonia	AF299157	AF299210	AF485631
Cunonia	*montana* Schltr.	*Bradford 609* (MO, NOU)	New Caledonia			AF485632
Cunonia	*pulchella* Brongn. & Gris	*Bradford 635* (MO, NOU)	New Caledonia			AF485633
Pancheria Brongn. & Gris (30)						
Pancheria	*brunhessii* Pampan.	*Bradford 616* (MO, NOU)	New Caledonia			AF485634
Pancheria	*engleriana* Schltr.	*Bradford 602* (MO, NOU)	New Caledonia	AF299158	AF299211	AF485635
Pancheria	*hirsuta* Vieill. ex Pampan.	*Bradford 610* (MO, NOU)	New Caledonia	AF299159	AF299212	AF485636
Pancheria	*phylliraeoides* Brongn. & Gris ex Guillaumin	*Bradford 620* (MO, NOU)	New Caledonia			AF485637
Pancheria	*reticulata* Guillaumin	*Bradford 618* (MO, NOU)	New Caledonia			AF485638
Vesselowskya Pamp. (2)						
Vesselowskya	*venusta* Rozefelds, R. W. Barnes & Pellow	*Bradford 879* (MO, NSW)	Australia	AF299160	AF299213	AF485639
Weinmannia L. (151)						
section *Leiospermum* D. Don (22)						
Weinmannia	*dichotoma* Brongn. & Gris	*Hopkins 5053* (MO, NOU)	New Caledonia	AF299142	AF299195	AF485595
Weinmannia	*parviflora* G. Forst.	*Bradford 914* (MO, PAP)	Society Islands			AF485596
Weinmannia	*racemosa* L. f.	*Bradford 936* (MO)	New Zealand (U.S.)			AF485597
Weinmannia	*raiatensis* J. W. Moore	*Bradford 927* (MO, PAP)	Society Islands	AF299141	AF299194	AF485598

Table 1. Continued.

Genus	Species (number of species in taxon)	Collection	Native origin (cultivated)	*trn*L c–d	*trn*L e–F	ITS1
Weinmannia	*samoensis* A. Gray	*Bradford 800* (MO)	Samoa	AF299143	AF299196	AF485599
Weinmannia	*silvicola* Sol. ex A. Cunn.	*Bradford 912* (MO, AKU)	New Zealand	AF299144	AF299197	AF485601
Weinmannia	*serrata* Brongn. & Gris	*Bradford 627* (MO, NOU)	New Caledonia			AF485600
Weinmannia	sp. nov. 1 (cf. Hopkins, 1998c)	*Bradford 837* (MO, BSIP)	Solomons			AF485594
Weinmannia	*vescoi* Drake	*Bradford 921* (MO, PAP)	Society Islands			AF485602
Weinmannia	*vitiensis* Seem.	*Hopkins 5041* (MO, P, SUVA)	Fiji			AF485603
section *Fasciculatae* Bernardi ex Hoogland & H. C. Hopkins (19)						
Weinmannia	*clemensiae* Steenis	*Hopkins 5011* (MO, KIN, P)	Malaysia	AF299147	AF299200	AF485612
Weinmannia	*exigua* A. C. Sm.	*Bradford 814* (MO, BSIP)	Solomons	AF299148	AF299201	AF485614
Weinmannia	*fraxinea* (1) Sm. ex D. Don	*Bradford 578* (MO, P, KEP)	Malaysia Peninsula	AF299149	AF299202	AF485613
Weinmannia	*fraxinea* (2) Sm. ex D. Don	*Hopkins 5001* (MO, SAN)	Borneo			AF485615
Weinmannia	*hooglandii* H. C. Hopkins & J. C. Bradford	*Bradford 579* (MO, P, KEP)	Malay Peninsula			AF485616
Weinmannia	*richii* A. Gray	*Hopkins 5023* (MO, P, SUVA)	Fiji			AF485617
section *Spicatae* Bernardi ex. J. C. Bradford (26)						
Weinmannia	*arguta* (Bernardi) J. C. Bradford	*Bradford 642* (MO, TAN)	Madagascar			AF485618
Weinmannia	*bojeriana* Tul.	*Bradford 639* (MO, TAN)	Madagascar			AF485619
Weinmannia	*humbertiana* Bernardi	*Bradford 695* (MO, TAN)	Madagascar			AF485620
Weinmannia	*marojejyensis* J. S. Mill. & J. S. Bradford	*Bradford 692* (MO, TAN)	Madagascar			AF485621
Weinmannia	*minutiflora* Baker	*Malcomber 2874* (MO, TAN)	Madagascar	AF299150	AF299203	AF485622
Weinmannia	*sanguisugarum* Bernardi	*Bradford 715* (MO, TAN)	Madagascar	AF299151	AF299204	AF485623
Weinmannia	*stenostachya* Baker	*Bradford 650* (MO, TAN)	Madagascar			AF485624
section *Inspersae* Bernardi ex J. C. Bradford (9)						
Weinmannia	*madagascariensis* DC. ex Ser.	*Bradford 653b* (MO, TAN)	Madagascar	AF299152	AF299205	AF485626
Weinmannia	*rutenbergii* Engl.	*Malcomber 2880* (MO, TAN)	Madagascar	AF299153	AF299206	AF485627
Weinmannia	sp. nov. 2	*Bradford 655* (MO, TAN)	Madagascar			AF485625
section *Weinmannia* L. (77)						
Weinmannia	*auriculata* Hieron.	*Bradford 547* (MO, USZ)	Bolivia			AF485605
Weinmannia	*bangii* Rusby	*Bradford 525* (MO, USZ)	Bolivia	AF299145	AF299198	AF485606
Weinmannia	*ellantantha* Diels	*Bradford 538* (MO, USZ)	Bolivia			AF485607
Weinmannia	*myrtifolia* Cuatrec.	*Bradford 745* (MO, COL)	Colombia			AF485608
Weinmannia	sp. nov. 3	*Bradford 435* (MO, PORT)	Venezuela			AF485604
Weinmannia	*tinctoria* Sm.	*D'Argent, s.n.* (MAU 22790)	Mauritius	AF299146	AF299199	AF485609
Weinmannia	*tomentosa* L. f.	*Bradford 751* (MO, COL)	Colombia			AF485610
Weinmannia	*trichosperma* Cav.	*Bradford 738* (MO)	Chile (U.S.)			AF485611

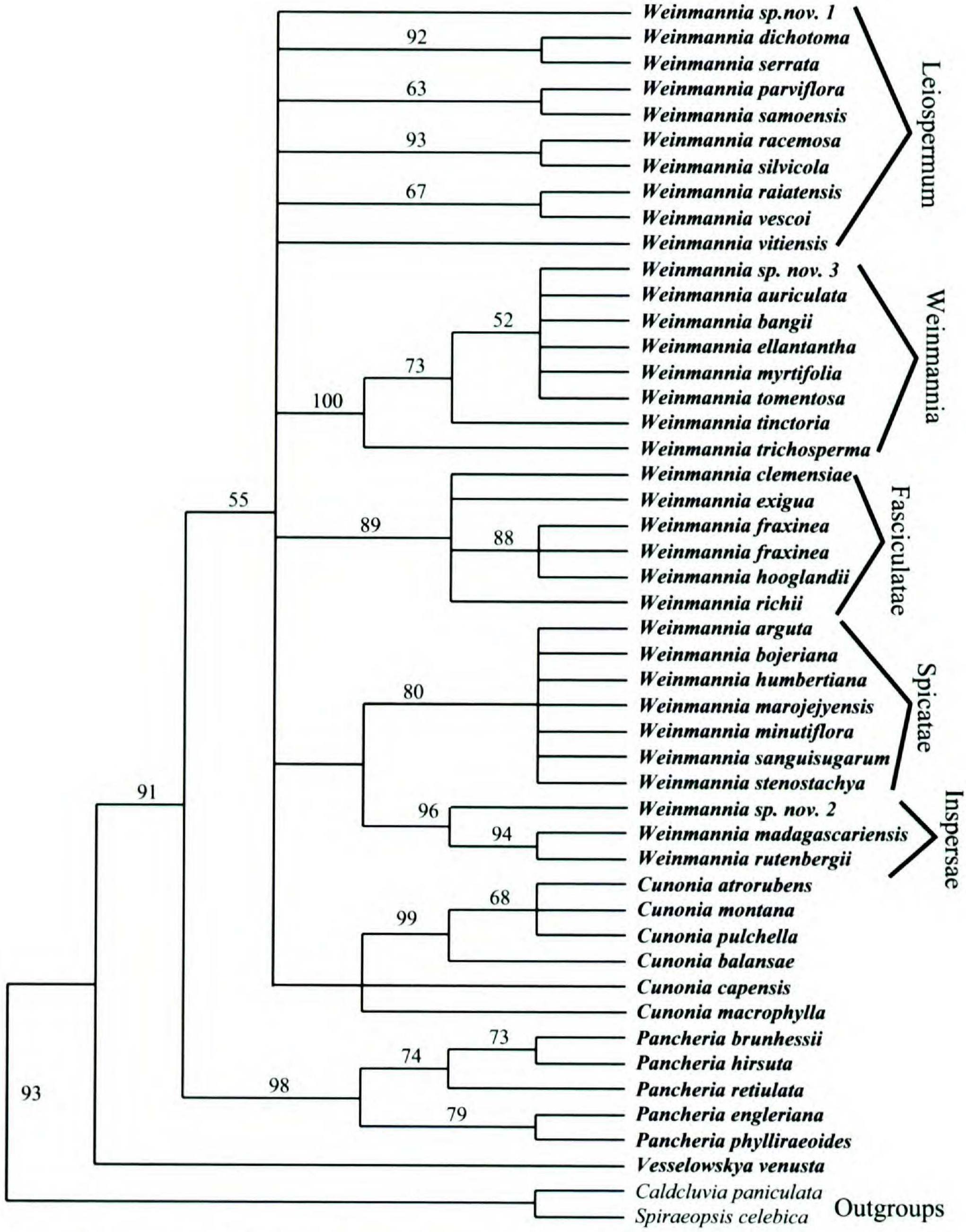

Figure 1. Strict consensus of >30,000 most parsimonious trees of ITS-1 sequences. "Fast" bootstrap values are above branches. Each section of *Weinmannia* is labeled to the right of the tree.

Discussion

In several respects, the phylogeny of the Cunoniae resulting from the combined analysis of ITS and *trn*L-F (Fig. 3) is highly congruent with previously published studies using morphological characters (Bradford, 1998). It differs primarily by showing that *Weinmannia* sect. *Weinmannia* is not nested within section *Fasciculatae*. Most significantly, the combined data support the monophyly of *Pancheria*, *Cunonia* (albeit weakly), and all five sections of *Weinmannia*. This analysis also suggests that Malagasy taxa form a clade. Although the combined data set does produce a highly resolved tree, "Fast" bootstrap and decay analyses do not give much support for internal nodes, indicating that relationships between major clades within Cunonieae are still poorly understood.

In contrast to the results of Bradford (1998), the molecular data sets do not uphold the monophyly

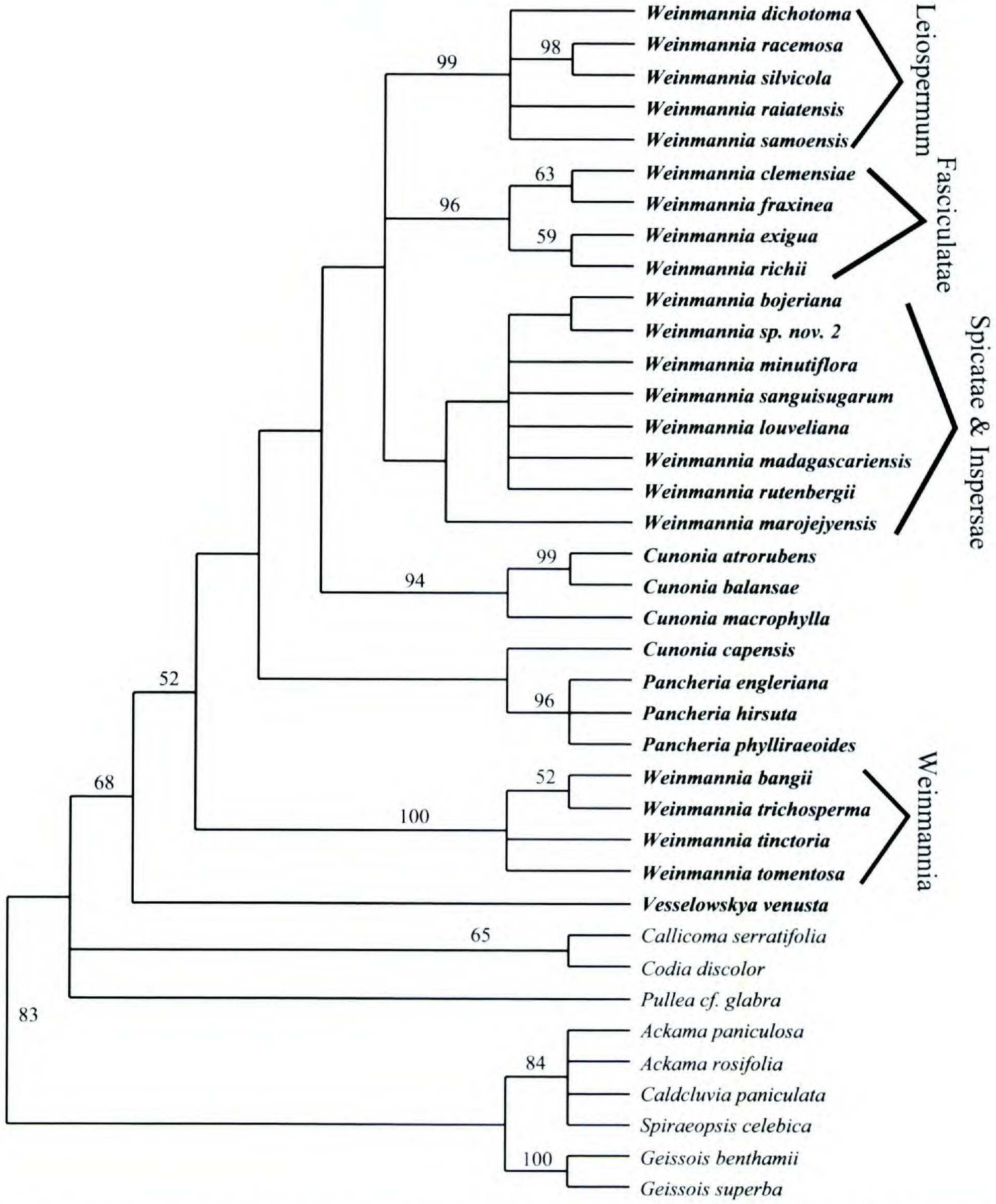

Figure 2. Strict consensus of *trn*L-*trn*F trees. "Fast" bootstrap values are above branches. Each section of *Weinmannia* is labeled. Cunonieae taxa are shown in bold type.

of *Weinmannia*. *Weinmannia* sect. *Weinmannia* has a very long branch and is placed as a sister group to *Cunonia* and other *Weinmannia* (Fig. 3). This long branch and the short internal nodes make it possible that these results are not dependable. To test whether this data set could statistically reject the hypothesis of a monophyletic *Weinmannia*, I built a constraint tree in MacClade (Maddison & Maddison, 1992) making *Weinmannia* monophyletic and enforced this topology in PAUP while reanalyzing the combined data set. A single tree of 390 steps was found, only one step more than the tree found in the unconstrained analysis. A Wilcoxon signed-rank test was then used to compare the most parsimonious tree with the monophyletic *Weinmannia* tree (Templeton, 1983; Mason-Gamer & Kellogg, 1996), and the null hypothesis of a monophyletic *Weinmannia* could not be rejected (N = 3, T = 2, $P = 0.56$). It would therefore be premature to consider *Weinmannia* paraphyletic based on this data.

Morphologically, *Weinmannia* is recognized easily by the presence of hairs on the seeds, which are lacking in other Cunonieae. Also, *Pancheria*, *Cunonia*, and *Vesselowskya* have winged seeds, but wings are lacking in *Weinmannia*. To account for

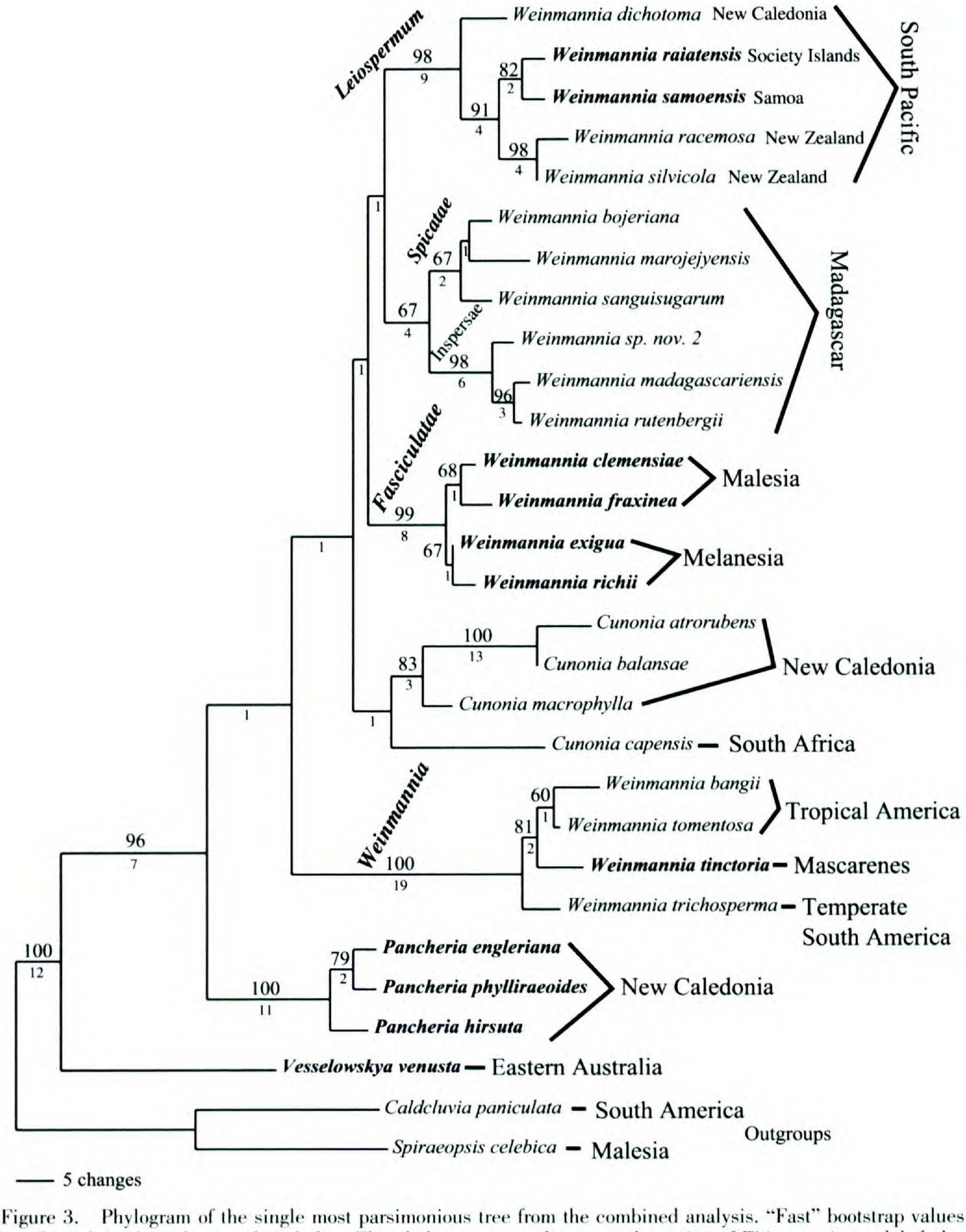

Figure 3. Phylogram of the single most parsimonious tree from the combined analysis. "Fast" bootstrap values are given above branches, decay values below. The clades corresponding to each section of *Weinmannia* are labeled, as is the geographic occurrence of species. Species placed in bold type have a dioecious breeding system; others are monoecious, usually with bisexual flowers.

these character states with a paraphyletic *Weinmannia*, two additional morphological steps are required: either seed hairs were gained twice and seed wings were lost twice (once on each of the two *Weinmannia* lineages), or a reversal of both characters occurred in *Cunonia* (Fig. 4). Other characters supporting the monophyly of *Weinmannia* have been found in micromorphological studies by R. Barnes (see Bradford & Barnes, 2001) in which multicellular hair bases were found in all sections of *Weinmannia*, but not in every species. These kinds of hairs have never been found in other genera of Cunoniaceae.

Although combined molecular data provide little

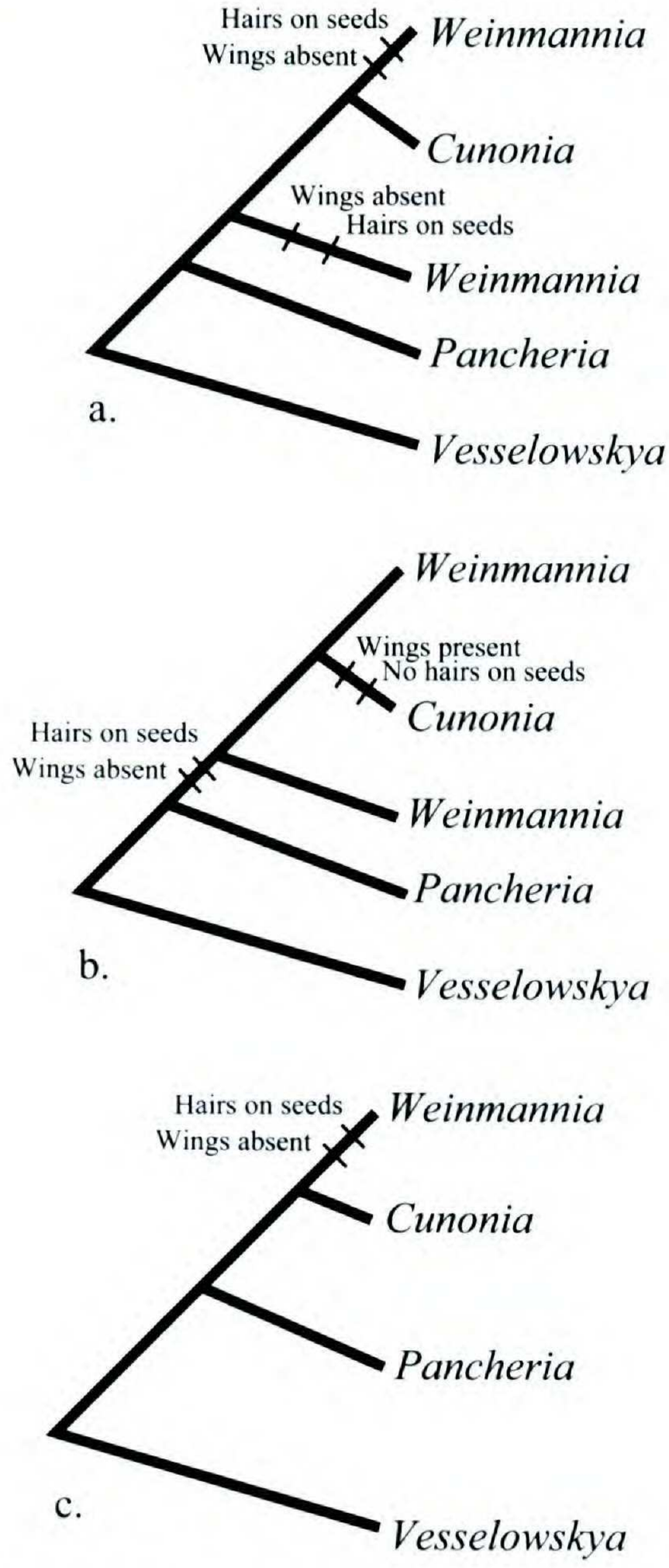

Figure 4. Alternative hypotheses for relationships within Cunonieae. —a & b. *Weinmannia* is paraphyletic with respect to *Cunonia*. This involves 389 steps in the combined molecular data set, and 4 more morphological ones. —c. *Weinmannia* is monophyletic. This involves 390 steps in the combined molecular data set, and 2 more morphological ones.

support for the cladistic relationships among *Pancheria*, *Cunonia*, and *Weinmannia* sections, new insights into relationships within genera and sections have emerged.

The South African species *Cunonia capensis* was recognized by Bradford (1998) as being morphologically similar to two very distinctive New Caledonian species, *C. macrophylla* and *C. schinziana*. All three species have larger flowers and fruits than other *Cunonia*, and similar inflorescence architectures composed of axillary pairs of stout racemes at the ends of stems. This inflorescence architecture is more reduced than the compound racemes typically found in other *Cunonia* (Hoogland et al., 1997). Morphological cladistic analyses showed this group of species to be a highly derived clade within *Cunonia*. In contrast, the combined molecular data make *Cunonia capensis* basal within *Cunonia*, and *C. macrophylla* basal within New Caledonian *Cunonia* (Fig. 3).

Most Cunoniaceae, including Cunonieae, are pollinated by small flying insects, especially bees. The distinctive features of *Cunonia capensis* and *C. macrophylla* may be due to their unusual pollination biology. Observations show their visitors include nectar-feeding birds that perch at the base of the raceme (Hopkins, pers. obs.; Coates Palgrave, 1983), which makes sense considering their relatively large flowers and simple, rigid inflorescence structure. The combined cladogram (Fig. 3) suggests that similarities between *Cunonia capensis* and *Cunonia macrophylla* may be plesiomorphic. Given that bird pollination is only known in one other Cunoniaceae genus (*Geissois*), it seems unlikely that bird-pollination was plesiomorphic during the origin of *Cunonia*, although it may be for the extant clade. Alternatively, bird pollination may be convergent in *Cunonia capensis* and *C. macrophylla*, but testing these hypotheses for ancient lineages is nearly impossible.

Although molecular systematics only gives weak support to the monophyly of *Cunonia*, the genus is morphologically distinct. At least two characters are shared only by species of *Cunonia*: fruits that have a circumbasal-acropetal dehiscence, a character unique in the family, and floral disks that are adnate to the base of the ovary, unlike any other Cunonieae (Bradford, 1998; Bradford & Barnes, 2001; Rozefelds et al., 2001).

The most well-supported clade in the analyses is that of *Weinmannia* sect. *Weinmannia*. This is the largest section in the genus and is disjunct between the Americas, where over 70 species occur, and the Mascarene Islands, where 2 species are endemic. The Mascarene species are distinguished by being dioecious, but otherwise are similar to American species (Bradford, 1998). Although the topology is not strongly supported, Mascarene species, represented here by *Weinmannia tinctoria*, are nested between *W. trichosperma*, from temperate forests of South America, and a clade of species from Neotropical montane forests.

Weinmannia trichosperma is apparently a remnant of a more ancient, temperate lineage within *Weinmannia* sect. *Weinmannia*—a lineage that macrofossils show may have once occurred in Tasmania as well (Carpenter & Buchanan, 1993). That a derived tropical clade of the section is disjunct between the neotropics and the Mascarenes suggests that interchange across the Atlantic and In-

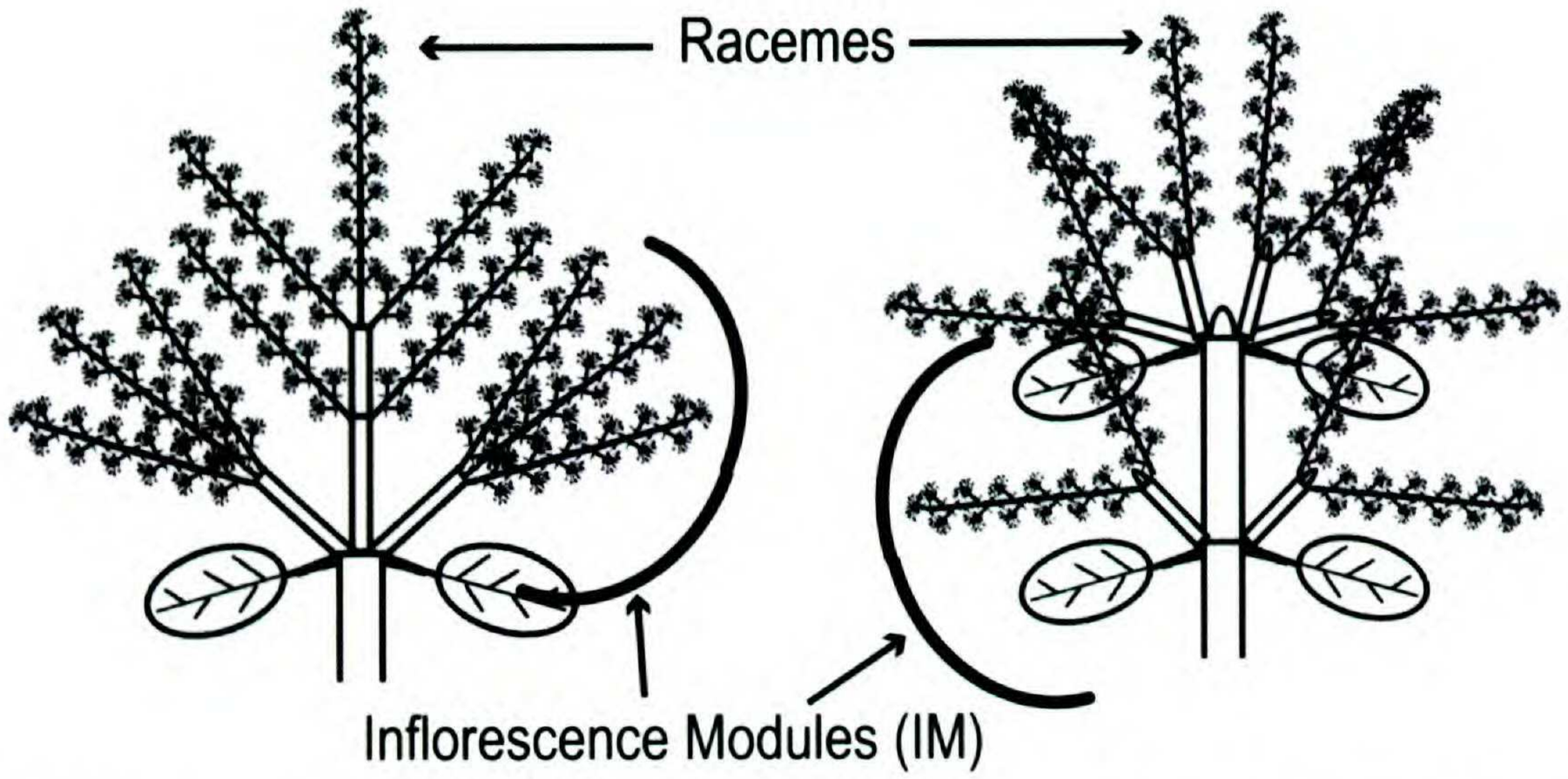

Figure 5. Two common inflorescence architectures in *Weinmannia* are shown; left, section *Leiospermum;* right, section *Fasciculatae.* Racemes, internodes, and meristems are organized in particular patterns to form higher order units called Inflorescence Modules (IMs), and IMs are organized in particular patterns along the main stem to comprise the Total Inflorescence.

dian Oceans has occurred more recently than interchange between temperate and tropical America. However, since only a few molecular characters support this unexpected relationship, additional data are required to test it.

In Bradford (1998), *Weinmannia* sect. *Fasciculatae* was paraphyletic with respect to a highly derived *Weinmannia* sect. *Weinmannia.* This view is overturned by the molecular evidence. *Weinmannia* sect. *Fasciculatae* has high bootstrap and decay values (Figs. 2–4). Missing from this study, however, is sequence data for *Weinmannia descombesiana,* an unusual species placed in section *Fasciculatae,* but with some traits suggestive of section *Leiospermum* (Hopkins, 1998b).

Weinmannia sect. *Leiospermum* is widely distributed in the South Pacific, from the Bismarcks to the Marquesas, and from Rapa to New Zealand (Hopkins, 1998a, 1998c; Hopkins & Florence, 1998). Bradford (1998) recognized three species groups within the section: New Caledonian species, New Zealand species, and other South Pacific species. The two species from New Zealand, *Weinmannia racemosa* and *W. silvicola,* each have distinctive and unique inflorescence architectures. South Pacific species outside of New Caledonia are dioecious. As a whole, New Caledonian species have no obviously derived features; however, *Weinmannia dichotoma* aborts its terminal meristem at every node. Although each individual data set is ambiguous, the combined analysis strongly supports the monophyly of section *Leiospermum* and places the New Caledonian clade sister to clades from the South Pacific and New Zealand (Fig. 3).

A well-supported phylogeny presents an opportunity to reevaluate some of the morphological characters I discussed in previous studies (Bradford, 1998). This earlier work emphasized inflorescence architecture and heterotopy, and some background on this is warranted here. The inflorescence in Cunonieae comprises nested sets of structures. The most familiar of these are the flower-bearing axes, raceme-like in most genera, but ball-shaped in *Pancheria* (Rozefelds et al., 2001). These flower-bearing axes, along with internodes and meristems, are typically arranged in repeated units I term Inflorescence Modules (IMs) (Fig. 5). The structure of IMs and their arrangement is highly variable among Cunonieae genera and *Weinmannia* sections (Fig. 6). This observation led to a system of coding inflorescence architecture based on principles of positional homology (see Bradford, 1998, for details).

Molecular systematics has confirmed the general perception from comparative morphology that inflorescence evolution represents heterotopy, and that these characters can be effectively coded for morphological cladistic analyses. For example, phylogenetic support for some clades in the morphological cladistic analysis was based mainly on these characters. The monophyly of *Weinmannia* sect. *Leiospermum* was supported by two characters of the inflorescence: having a sequential arrangement of metamers within an IM, and having the largest IMs in the terminal (i.e., acrotonic) position (Figs. 5, 6, 7).

Furthermore, improved support for clades within section *Leiospermum* can be used to make specific statements about the pattern of heterotopic changes. From the generalized inflorescence form of section *Leiospermum* (see top and bottom left diagrams

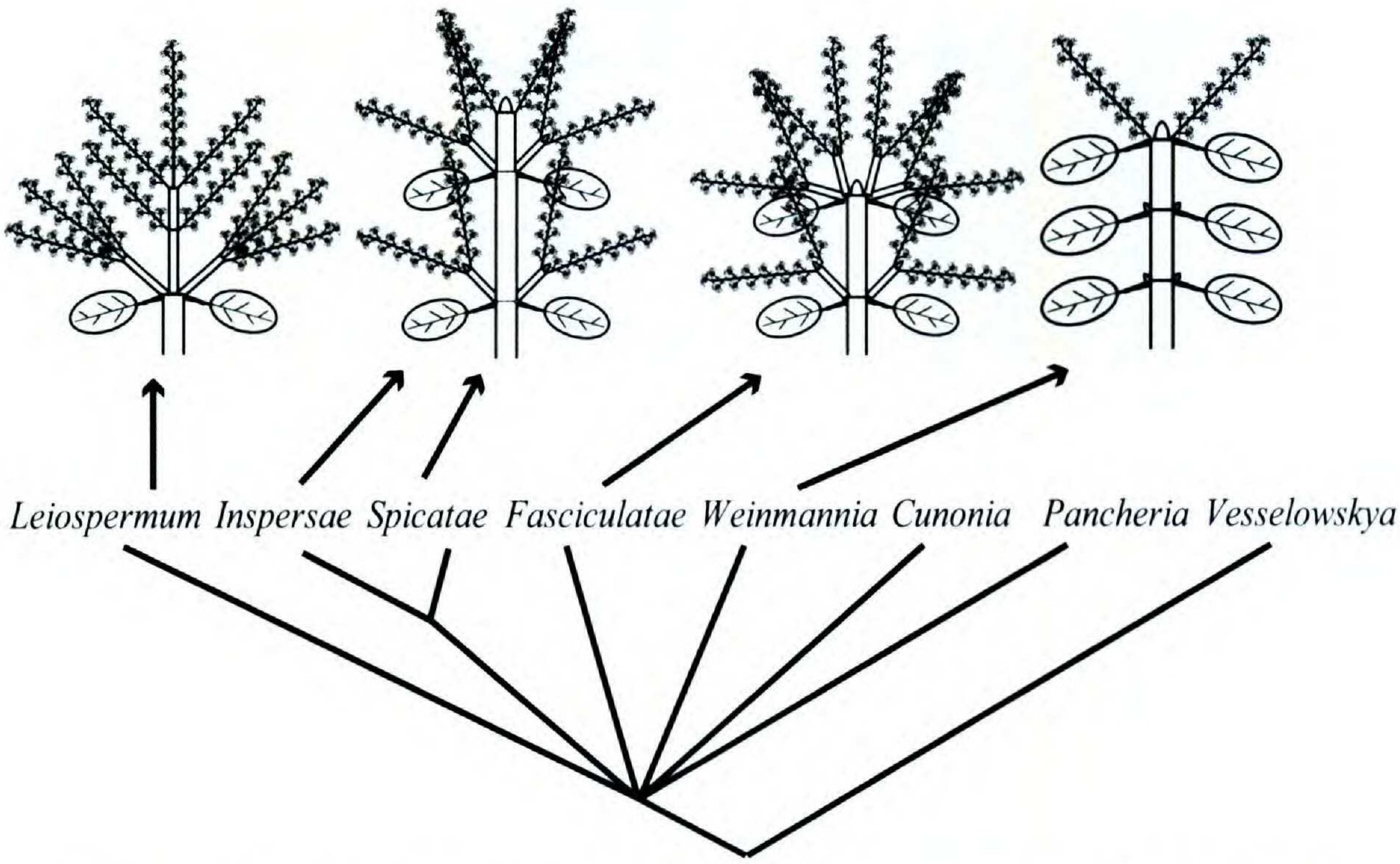

Figure 6. The general breadth of inflorescence diversity in *Weinmannia* is illustrated and linked to taxonomy and phylogeny. The phylogeny is based upon Figure 3, but clades having no bootstrap support and a decay value of only 1 were collapsed. *Cunonia* and *Pancheria* inflorescences are typically similar to sections *Inspersae* and *Spicatae*, although *C. macrophylla* and *C. capensis* are similar to section *Weinmannia*. *Vesselowskya* inflorescences display similarities to both sections *Fasciculatae* and *Leiospermum*. For detailed illustrations of inflorescence diversity in Cunonieae see Bradford (1998, 2001), Hoogland et al. (1997), the publication series of Hopkins (1998), and Rozefelds et al. (2001).

of Fig. 7), it is clear that species from New Zealand (*Weinmannia racemosa* and *W. silvicola*) have derived heterotopic changes in their inflorescences (see right diagrams of Fig. 7). *Weinmannia racemosa* has regained vegetative growth beyond the inflorescence with a terminal vegetative bud, a reversal to the plesiomorphic state found in other sections of *Weinmannia* (Fig. 6). This species has also lost the development of lateral inflorescence modules. The other species from New Zealand, *Weinmannia silvicola*, has developmental asymmetry; although it does not always produce lateral IMs, when present, they develop only from one axillary bud. Furthermore, *Weinmannia silvicola* is the only species to produce sequential metamers within an IM and then abort the apical meristem. Other species, such as *Weinmannia dichotoma* from New Caledonia (see left diagram in Fig. 7), abort the apical meristem at the first metamer within an IM. Although abortion of the apical meristem occurs as part of normal variation among IMs within many *Weinmannia* plants in the South Pacific, the fixation of this trait is apparently derived within *Weinmannia dichotoma*. (See also Hopkins, 1998a, 1998c; Hopkins & Florence, 1998.)

Without a more resolved phylogeny it is difficult to re-evaluate other characters discussed previously (Bradford, 1998), such as dioecy. Although the strong phylogenetic hypothesis for *Weinmannia* sect. *Leiospermum* indicates that dioecy evolved once within this clade (Fig. 3), it is difficult to discern the general pattern of breeding system evolution within Cunonieae (see also Bradford, 1998; Rozefelds et al., 2001).

Conclusions

Molecular systematics has enabled us to confidently delineate some major lineages within Cunonieae and provided sufficient resolution in some clades to re-examine inflorescence evolution. Unfortunately, little is known still about how Cunonieae genera and *Weinmannia* sections relate, except for the basal position of *Vesselowskya* (Fig. 6). Until a better phylogenetic hypothesis is available, it is best to retain the genera as currently circumscribed, despite questions about the monophyly of *Weinmannia*. Remaining to be addressed is character evolution for many traits that vary among genera of Cunonieae and sections of *Weinmannia*, although evolution within some genera and sections has been clarified.

This work is the first in-depth attempt to understand the phylogeny of Cunonieae. While taxon sampling was broad, the number of sequenced loci was likely insufficient to resolve all nodes. Ques-

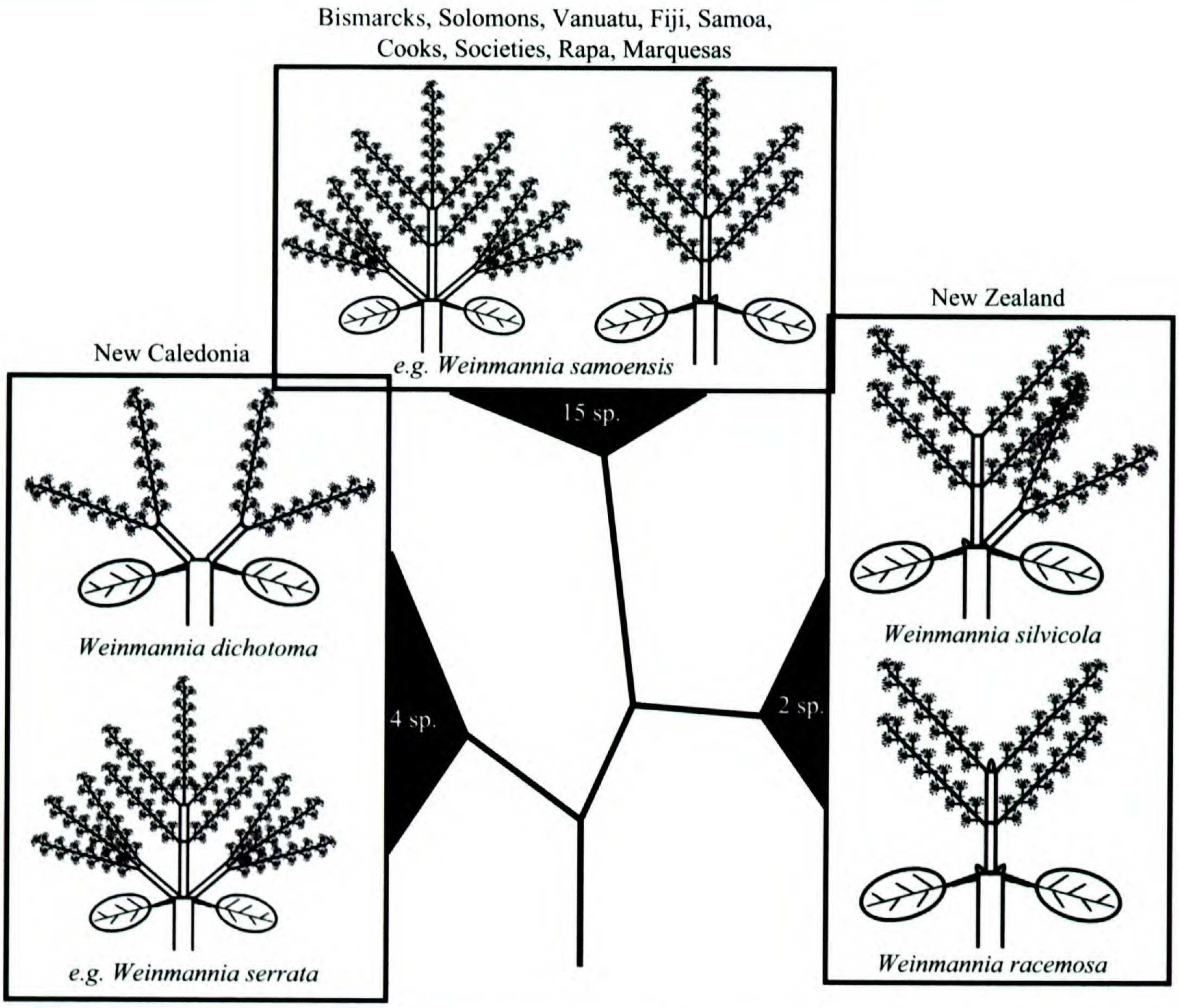

Figure 7. Relationships among clades within *Weinmannia* sect. *Leiospermum* based on the analyses presented here. The number of species and location of each clade is labeled. Some common forms of inflorescence architecture are shown, and the most unusual species, *Weinmannia silvicola*, *W. racemosa*, and *W. dichotoma*, are highlighted.

tions about character evolution may best be studied by comparing variation within species, sections, and genera rather than at the tribal level. For example, Malagasy *Weinmannia* have the richest variety of inflorescence architecture, sympatric *Cunonia* species have a diversity of floral coloration and scents, and in many *Weinmannia* species from Malesia and the South Pacific dioecy is "leaky" with morphologically male, female, and bisexual flowers found within a single population or plant. Most importantly, studies on the spatial ecology of species are almost totally lacking and would be useful for effective conservation measures.

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Appendix 1

The following are correct orthographies (following Greuter et al., 2000, ICBN Art. 21.2) and original publications of all five sections of *Weinmannia*. Types are also designated here for two sections.

Weinmannia L., Syst. Nat., ed. 10, 2: 997, 1005, 1367. 1759, nom. cons. TYPE: *Weinmannia pinnata* L.

Weinmannia sect. *Simplicifoliae* Bernardi, Candollea 18: 289. 1963, nom. invalid., sine typo.

Weinmannia sect. **Leiospermum** (D. Don) Engl., Nat. Pflanzenfam. 3 (2a): 101. 1891. *Leiospermum* D. Don, Edinburgh New Philos. J. 9: 91. 1830. TYPE: *Weinmannia racemosa* L.f., Suppl. 227. 1781. (lectotype, designated by H. C. Hopkins, 1998a: 21).

Weinmannia sect. *Racemosae* Bernardi, Bot. Jahrb. Syst. 83: 132, 185. 1964.

Weinmannia sect. **Fasciculatae** Bernardi ex Hoogland & H. C. Hopkins, Adansonia, sér. 3: 21. 1998. TYPE: *Weinmannia fraxinea* (D. Don) Miq.

Weinmannia sect. **Inspersae** Bernardi ex J. C. Bradford, **sect. nov.** *Weinmannia* sect. *Inspersae* Bernardi, Bot. Jahrb. Syst. 83: 132, 143. 1964, nom. invalid., sine typo. TYPE: *Weinmannia madagascariensis* DC. This section was originally published without a type species.

Weinmannia sect. **Spicatae** Bernardi ex J. C. Bradford, **sect. nov.** *Weinmannia* sect. *Spicatae* Bernardi, Bot. Jahrb. Syst. 83: 132. 1964, nom. invalid., sine typo. TYPE: *Weinmannia bojeriana* Tul. This section was originally published without a type species.